Guidance for Industry

Revised Recommendations for the Invalidation of Test Results When Using Licensed and 510(k) Cleared Bloodborne Pathogen Assays to Test Donors

DRAFT GUIDANCE

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Draft - Not for Implementation

Guidance for Industry[1]:

REVISED RECOMMENDATIONS FOR THE INVALIDATION OF TEST RESULTS WHEN USING LICENSED AND 510(k) CLEARED BLOODBORNE PATHOGEN ASSAYS TO TEST DONORS

I. INTRODUCTION

To reduce infectious disease transmission by blood and blood products, donor samples from blood donations are tested for markers of pathogenic bloodborne infections, including antibodies, antigens, and nucleic acids that may indicate the presence of etiologic agents such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C Virus (HCV), human T-cell lymphotropic virus (HTLV), cytomegalovirus (CMV), syphilis, and others. The validity of screening and supplemental (confirmatory) test assay results is determined by the performance of test kit manufacturer supplied reagents labeled as "controls", used in accordance with the test kit instructions. Historically, FDA has not required additional assay-specific quality control procedures beyond those documented in the test kit package inserts. FDA recommends implementation of quality assurance and quality control procedures that involve use of external control reagents beyond those provided by the test kit manufacturer in order to contribute to overall testing accuracy and therefore to blood safety.

II. BACKGROUND

On May 28, 1992, September 25, 1992, September 26, 1996, and December 13, 1996, the Blood Products Advisory Committee (BPAC) discussed in open public meetings circumstances under which test results may be invalidated, including actions based on the use of additional quality control reagents in donor testing. These discussions included general aspects of quality assurance related to bloodborne pathogen testing, invalidation procedures based on test kit product insert requirements, and additional procedures to invalidate test results based on criteria

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[1]This guidance document represents FDA's current thinking on invalidating test results based on the Clinical Laboratory Improvement Act of 1988 (CLIA) required control reagents. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

developed by the user. FDA issued guidance on this subject on January 3, 1994 (1). In an effort to further clarify FDA's guidance to ensure compliance with the Clinical Laboratory Improvement Act of 1988 (CLIA), at the December 13, 1996 BPAC meeting, the FDA, the Centers for Disease Control and Prevention (CDC), and the Health Care Financing Administration (HCFA) discussed elements of CLIA. CDC articulated the differences between a calibrating reagent and a reagent serving the function of an assay control. Based on CLIA regulations, CDC stated that if a test kit supplied "control" reagent is used to calculate the assay cutoff, regardless of its container label title, the reagent serves the function of a calibrator and may *not* serve simultaneously as a control reagent. In such instances, an additional reagent should be included in the assay run to meet CLIA control reagent requirements.

For example, if a test kit includes two reagents labeled as negative control and positive control respectively, and the negative control reagent is used to calculate the assay cutoff, the negative control serves as a calibrator and may not also serve as an assay control reagent. Since CLIA regulations require use of a minimum of two controls each day of testing, a negative control from an external source should be included in the run to meet CLIA requirements. Such external controls may be developed in-house or procured, and the blood establishment should develop acceptance/rejection criteria for their use. For further information, blood establishments should refer to CLIA regulations under Title 42, Code of Federal Regulations, Part 493, or contact the State Survey Agency in the state where the laboratory is located or the accrediting organization with which the laboratory is affiliated.

It is important to understand that the CLIA regulations specify requirements for donor testing that go beyond the test kit package insert instructions. The test kit package insert instructions should continue to be followed in order for runs to be considered valid for donor testing. Accordingly, externally supplied reagents should not be substituted for test kit supplied reagents to calculate the assay cutoff.

As a result of discussions held during BPAC meetings, and additional discussions among CDC, HCFA, and FDA, FDA has developed revised recommendations for blood establishments, integrating current CLIA provisions for invalidating test results based on CLIA designated control reagents. This guidance document, once finalized, is intended to supersede the January 3, 1994 guidance document (1). These procedures should be part of blood establishments' comprehensive quality assurance programs (2) and documented in blood establishments' Standard Operating Procedures (SOPs).

Because blood safety relies significantly on the accuracy of testing, user-developed acceptance/rejection rules should be designed ***only*** with the objective of enhancing blood safety. Accordingly, rules developed by blood establishments to meet either CLIA requirements or other in-house developed requirements should only be used to invalidate ***nonreactive*** results. **Reactive results obtained from a screening test or confirmatory/supplemental test run that satisfies the test kit's criteria for acceptance should not be invalidated**. Such reactive results remain as the initial test of record, and, in the case of a screening test, these specimens should be retested in duplicate as specified in the test kit manufacturer's instructions. The duplicate retest (duplicate rescreen) is designed to reduce the probability of obtaining a false negative result for a truly positive, but borderline reactive specimen. A donor sample with an invalidated nonreactive result may be retested singly.

III. SPECIFIC RECOMMENDATIONS

These recommendations for procedures to invalidate test results, based on: 1) test kit package insert rejection criteria; and 2) CLIA provisions, are applicable to all blood establishments and should be included in their Standard Operating Procedures.

A. CLIA Requirements for Control Reagents

CLIA regulations require control reagents to be used according to 42 CFR 493. If a bloodborne pathogen test kit uses any of its manufacturer supplied reagents to serve as a calibrator function, *i.e.*, either or both of the test kit controls (negative or positive) are used to calculate the assay cutoff, then CLIA regulations require that (an) additional "control" reagent(s) be included in each run (see table below). Such reagents may be procured or developed in-house. In any case, prior to placing the additional controls in routine use, each lot of such reagents should have: 1) a known dating period, *i.e.,* validated stability (supplied by a control reagent manufacturer or validated by the user on in-house developed control reagent); and 2) known performance parameters, *i.e.,* specifications for acceptance. Prior to implementation, additional control reagents should be qualified to minimize possible incompatibilities that may exist with particular test kits.

TEST KIT REAGENT(S)	USED IN CALCULATION OF THE CUTOFF?	ADDITIONAL CONTROL REAGENT REQUIRED
Negative Control	Yes	Yes (Negative Control)
Negative Control	No	No
Positive Control	Yes	Yes (Positive Control)
Positive Control	No	No
Positive and Negative Control	Yes	Yes (Positive and Negative Controls)

B. Invalidation of Test Results Based on Test Kit Package Insert

All results, both reactive and non-reactive, must be invalidated when it has been determined that the assay, or part of the assay, has not been performed according to the test kit package insert instructions, (*e.g*., improper procedure, compromised reagent,

faulty equipment) or that the test kit package insert defined run acceptance criteria have not been met, (*e.g.*, controls out of specified range) [21 CFR 606.65(e)]. Whenever such situations occur, test results should be invalidated and the subsequent valid assay becomes the initial test of record. The determination of nonadherence to the test kit package insert requirements may be a result of an investigation into an unexplained discrepancy, a routine review of the assay data, or concerns raised by a blood establishment's quality control procedures. For example, an investigation of procedures may be performed in response to unexpected external control reagent values or failure to meet the blood establishment's additional statistical acceptance criteria for rate of reactivity.
Documentation of each incident of invalidation should include the basis for invalidation, the details of an investigation, including records of supervisory oversight, the outcome of the investigation, and, if indicated, any corrective action taken. All of these actions should be taken prior to repeat testing of donor samples.

C. Invalidation of Test Results Based on CLIA Regulations

When the test kit package insert instructions have been met, but CLIA control requirements have ***not*** been met: 1) ***any reactive results should not be invalidated and 2) non-reactive results should be invalidated.*** All specimens giving initially reactive results should be re-tested in duplicate, or as the package insert directs [21 CFR 606.65(e)]. If either of the repeated duplicates is reactive, the unit should be classified as repeatedly reactive and no further repeat testing should be performed. Specimens giving repeatedly reactive test results then should be further assayed and reported as recommended in FDA Memoranda to Blood Establishments (3, 4, 5, 6, 7, 8, 9) regarding viral marker testing of donations. Invalidated non-reactive specimens should be re-tested singly and those results become the initial test of record.

Invalidation criteria pursuant to CLIA requirements should be based on site-user developed rules, *e.g.*, population reactive prevalence data, or standard deviation based limits, which may be derived from generally accepted clinical laboratory quality control algorithms.
Documentation for ***all*** incidents of invalidation should include the basis for invalidation, the details of an investigation, including records of supervisory oversight, the outcome of

the investigation, and, if indicated, any corrective action taken. All of these actions should be taken prior to repeat testing of donor samples.

Reactive results should only be invalidated when an assay run either fails to meet test kit package insert acceptance criteria or the assay was not performed in accordance with the test kit package insert. Accordingly, invalidation of reactive test results should not be based solely on either CLIA required quality control reagent performance or site supplemental quality control rules. Examples of circumstances when reactive results should not be invalidated include CLIA required control reagent values exceeding site established quality control acceptance limits, or reactive rates exceeding site defined upper limits.

IV. REFERENCES

1. FDA Recommendations for the Invalidation of Test Results When Using Licensed Viral Marker Assays to Screen Donors, 3 January 1994, Center for Biologics Evaluation and Research, FDA.

2. Guideline for Quality Assurance in Blood Establishments, 11 July 1995, Center for Biologics Evaluation and Research, FDA.

3. Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV), 23 April 1992, Center for Biologics Evaluation and Research, FDA.

4. Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV), 5 August 1993, Center for Biologics Evaluation and Research, FDA.

5. Additional Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV), 16 May 1996, Center for Biologics Evaluation and Research, FDA.

6. Recommendations for the Management of Donors and Units that are Initially Reactive for Hepatitis B Surface Antigen (HBsAg), 2 December 1987, Center for Biologics Evaluation and Research, FDA.

7. Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products, 23 April 1992, Center for Biologics Evaluation and Research, FDA.

8. FDA Recommendations Concerning Testing for Antibody to Hepatitis B Core Antigen (Anti-HBc), 10 September 1992, Center for Biologics Evaluation and Research, FDA.

9. HTLV-I Antibody Testing, 29 September 1989, Center for Biologics Evaluation and Research, FDA.